(54) Title: METHOD AND APPARATUS FOR MULTI-SPECTRAL ANALYSIS IN NONINVASIVE NIR SPECTROSCOPY

(57) Abstract

A method and apparatus are described for determining the concentration of an analyte present in a sample using multi-spectral analysis in the near infrared range. Incident radiation containing a plurality of distinct, nonoverlapping regions of wavelengths in the range of approximately 1100 to 3500 nm is used to scan a sample. Diffusively reflected radiation emerging from the sample is detected, and a value indicative of the concentration of the analyte is obtained using an application of chemometrics techniques. Information obtained from each nonoverlapping region of wavelengths can be cross-correlated in order to remove background interferences.

5        METHOD AND APPARATUS FOR MULTI-SPECTRAL
         ANALYSIS IN NONINVASIVE NIR SPECTROSCOPY

<u>Technical Field</u>:
              The present invention relates to a method
10    and apparatus for determining the concentration of a
      target analyte in a sample using multi-spectral
      analysis.  The invention finds application in a wide
      range of chemical analyses, particularly in
      noninvasive spectrophotometric analysis of blood
15    analytes.

<u>Background of the Invention</u>:
              The measurement of the concentration of
      blood constituents finds application in a variety of
20    procedures for the diagnosis and treatment of
      conditions and disease in human subjects.  One
      important application is in the measurement of blood
      glucose.  Specifically, the concentration of blood
      glucose should be monitored on a periodic basis in
25    persons suffering from diabetes, and in insulin-
      dependent or Type I diabetes, it is often necessary or
      desirable to monitor blood glucose several times a
      day.  Further, the measurement of blood cholesterol
      concentrations provides important information in the
30    treatment or prevention of persons suffering from
      coronary artery disease, and the measurement of other
      organic blood analytes, such as bilirubin and alcohol,
      is important in various diagnostic contexts.
              The most accurate and widely practiced
35    method of obtaining blood analyte concentrations
      involves the extraction of blood from a patient, which
      blood is then analyzed, either in a laboratory using

                              -1-

highly accurate and sensitive assay techniques, or by
the use less accurate self-testing methods. In
particular, traditional blood glucose monitoring
methods require the diabetic to draw a blood sample
5    (e.g., by a finger-tip lance) for each test and to
read the glucose level using a glucometer (a
spectrophotometer that reads glucose concentrations)
or a colorimetric calibration method. Such invasive
blood extractions create a painful and tedious burden
10   to the diabetic and expose the diabetic to the
possibility of infection, particularly in light of the
frequency of testing which is necessary. These
considerations can lead to an abatement of the
monitoring process by the diabetic.

15        Accordingly, there is a recognized need in
the art for a simple and accurate method and device
for noninvasively measuring blood analyte
concentration, particularly in the context of blood
glucose monitoring by diabetics. One approach to the
20   problem involves the use of traditional methods of
near infrared (near-IR, or "NIR") analysis, wherein
the measurement of absorbance at one or more specific
wavelengths is used to extract analyte-specific
information from a given sample.

25        Near-IR absorbance spectra of liquid samples
contain a large amount of information about the
various organic constituents of the sample.
Specifically, the vibrational, rotational and
stretching energy associated with organic molecular
30   structures (e.g., carbon-carbon, carbon-hydrogen,
carbon-nitrogen and nitrogen-hydrogen chemical bonds)
produces perturbations in the near-IR region which can
be detected and related to the concentration of
various organic constituents present in the sample.
35   However, in complex sample matrices, near-IR spectra
also contain an appreciable amount of interferences,
due in part to similarities of structure amongst

analytes, relative levels of analyte concentration,
interfering relationships between analytes and the
magnitude of electronic and chemical "noise" inherent
in a particular system.  Such interferences reduce the
5    efficiency and precision of measurements obtained
using near-IR spectrometry to determine the
concentration of liquid sample analytes.  However, a
number of near-IR devices and methods have been
described to provide noninvasive blood analyte
10   determinations.

U.S. Patent No. 5,360,004 to Purdy et al.
describes a method and apparatus for the determination
of blood analyte concentrations, wherein a body
portion is irradiated with radiation containing two or
15   more distinct bands of continuous-wavelength incident
radiation.  Purdy et al. emphasize filtration
techniques to specifically block radiation at the two
peaks in the NIR absorption spectrum for water,
occurring at about 1440 and 1935 nm.  Such selective
20   blocking is carried out in order to avoid a heating
effect that may be due to the absorption of radiation
by water in the body part being irradiated.

By contrast, U.S. Patent No. 5,267,152 to
Yang et al. describes noninvasive devices and
25   techniques for measuring blood glucose concentration
using only the portion of the IR spectrum which
contains the NIR water absorption peaks (e.g., the
"water transmission window," which includes those
wavelengths between 1300 and 1900 nm).  Optically
30   controlled light is directed to a tissue source and
then collected by an integrating sphere.  The
collected light is analyzed and blood glucose
concentration calculated using a stored reference
calibration curve.

35   Devices have also been described for use in
determination of analyte concentrations in complex
samples.

For example, U.S. Patent No. 5,242,602 to
Richardson et al. describes methods for analyzing
aqueous systems to detect multiple active or inactive
water treating components.  The methods involve
5    determination of the absorbance or emission spectrum
of the components over the range of 200 to 2500 nm,
and application of chemometrics algorithms to extract
segments of the spectral data obtained to quantify
multiple performance indicators.

10              U.S. Patent No. 5,252,829 to Nygaard et al.
describes a method and apparatus for measuring the
concentration of urea in a milk sample using an
infrared attenuation measuring technique.
Multivariate techniques are carried out to determine
15   spectral contributions of known components using
partial least squares algorithms, principal component
regression, multiple linear regression or artificial
neural network learning.  Calibration is carried out
by accounting for the component contributions that
20   block the analyte signal of interest.  Thus, Nygaard
et al. describe a technique of measuring multiple
analyte infrared attenuations and compensating for the
influence of background analytes to obtain a more
accurate measurement.

25              U.S. Patent No. 4,975,581 to Robinson et al.
describes a method and apparatus for determining
analyte concentration in a biological sample based on
a comparison of infrared energy absorption (i.e.,
differences in absorption at several wavelengths)
30   between a known analyte concentration and a sample.
The comparison is performed using partial least
squares analysis or other multivariate techniques.

             U.S. Patent No. 4,882,492 to Schlager
describes a method and apparatus for non-invasive
35   determination of blood analyte concentrations.
Modulated IR radiation is directed against a tissue
sample (e.g., an ear lobe) and either passed through

                              -4-

the tissue or impinged on a skin surface where it is
spectrally modified by a target analyte (glucose).
The spectrally modified radiation is then split,
wherein one portion is direct d thr ugh a negativ
5    correlation cell and another through a reference cell.
Intensity of the radiation passing through the cells
are compared to determine analyte concentration in the
sample.

U.S. Patent No. 4,306,152 to Ross et al.
10   describes an optical fluid analyzer designed to
minimize the effect of background absorption (i.e.,
the overall or base level optical absorption of the
fluid sample) on the accuracy of measurement in a
turbid sample or in a liquid sample which is otherwise
15   difficult to analyze.  The apparatus measures an
optical signal at the characteristic optical
absorption of a sample component of interest and
another signal at a wavelength selected to approximate
background absorption, and then subtracts to reduce
20   the background component of the analyte-dependent
signal.

The accuracy of information obtained using
the above-described methods and devices is limited by
the spectral interference caused by background, i.e.,
25   non-analyte, sample constituents that also have
absorption spectra in the near-IR range.  Appreciable
levels of background noise represent an inherent
system limitation, particularly when very little
analyte is present.  In light of this limitation,
30   attempts have been made to improve signal-to-noise
ratios, e.g., by avoiding water absorption peaks to
enable the use of increased radiation intensity, by
reducing the amount of spectral information to be
analyzed, or by using subtraction or compensation
35   techniques based on an approximation of background
absorption.  Although such techniques have provided
some improvement, there remains a need to provide a

-5-

method and apparatus capable of rend ring a more
precise determination of the concentration of analytes
in a liquid matrix, particularly in the context of
blood glucose monitoring.

5

Disclosure of the Invention

       Accordingly, it is a primary object of the
invention to address the above-described needs in the
art, by providing a method of determining the

10   concentration of an analyte present in a sample having
a varying background matrix and possibly having
substantial component interferences as well.  The
method accounts for the  similarity of structures
among various components present in the sample, the

15   relative magnitude of the analyte concentration and
spectral interferences provided by various sample
components and instrumentation variances.

       The method generally involves: (1)
identifying several distinct, nonoverlapping regions

20   of wavelengths in the near-IR range, which have high
correlation to the concentration of the analyte; (2)
irradiating a sample with incident radiation
containing those regions in order to obtain radiation
that has been spectrally attenuated as a result of

25   interaction with sample constituents; (3) detecting
the spectrally attenuated radiation; (4) measuring the
intensity of the spectrally attenuated radiation at a
wavelength in nonoverlapping regions of wavelengths;
and (5) correlating the measurements to obtain a value

30   indicative of the concentration of the analyte.

       It is also an object of the invention to
provide a spectrophotometric apparatus for determining
the concentration of an analyte present in a sample
having a varying background matrix and substantial

35   component interferences.  The apparatus is used in a
multi-spectral analysis to obtain spectral information
containing analyte-specific signals as well as signals

-6-

related to instrument background noise and int rfering
sp ctral information. Chemometrics techniques are
used to c nfigure filter elements capable f nhancing
the corr lation of analyte-specific information with

5    the concentration of the analyte and to derive system
algorithms capable of determining analyte
concentration values.

In one aspect of the invention, an apparatus
is provided which includes a specialized optical

10   transfer cell that is capable of enhancing the
correlation of analyte-specific information with the
concentration of the analyte. The specialized optical
transfer cell contains a positive correlation filter
adapted to selectively emphasize wavelengths having

15   high correlation with the concentration of a selected
analyte. The emphasized wavelengths are communicated
to a means for receiving the information and
converting the same into a signal representative of
the intensity of the wavelength.

20

Brief Description of the Figures
Figure 1 is a diagrammatic representation of
an apparatus constructed according to the invention.
Figure 2 is a diagrammatic representation of

25   a correlation spectrometer apparatus constructed
according to the invention.
Figure 3 is a graph illustrating time-
dependent scans taken during an *in vivo* glucose
tolerance study.

30           Figure 4 depicts in graph form the results
obtained from a noninvasive determination of blood
glucose concentration conducted using the method of
the invention.


35   Modes for Carrying Out the Invention
Before the invention is described in detail,
it is to be understood that this invention is not

-7-

limited to the particular component parts of the
devices or methods described, as such may vary. It is
also to be understood that the t rminology used herein
is for purposes of describing particular embodiments
5    only, and is not intended to be limiting. It must be
noted that, as used in the specification and the
appended claims, the singular forms "a," "an" and
"the" include plural referents unless the context
clearly dictates otherwise. Thus, for example,
10   reference to "an analyte" includes mixtures of
analytes, reference to "an optical transfer cell"
includes two or more optical transfer cells, "a means
for reflectively transmitting radiation" includes two
or more such means, "a wavelength" includes two or
15   more wavelengths, "a chemometrics algorithm" includes
two or more algorithms, and the like.

        In this specification and in the claims
which follow, reference will be made to a number of
terms which shall be defined to have the following
20   meanings:

        "Chemometrics" relates to the application of
mathematical, statistical and pattern recognition
techniques in chemical analysis applications. *See*,
*e.g.*, Brown et al. (1990) *Anal. Chem.* 62:84-101.
25   Chemometrics is practiced herein in the context of
developing and using noninvasive diagnostic
instrumentation that employs advanced signal
processing and calibration techniques. Signal
processing is used to improve the accessibility of
30   physically significant information in analytical
signals. Examples of signal processing techniques
include Fourier transformation, first and second
derivatives, and digital or adaptive filtering.

        In the context of chemometrics,
35   "calibration" refers to the process of relating data
measurements to a chemical concentration for the
purpose of quantification. Particularly, statistical

-8-

calibrations using chemometric methods can be used to
extract specific information from a complex set of
data.  Such methods  f calibration include linear
regression, multiple-linear regression, partial linear
5     regression, and principal components analysis.  In
other applications, calibrations can be carried out
using artificial neural networks, genetic algorithms
and rotated principal components analysis.

            Instrumentation that detects information for
10    one or more constituents in a complex chemical matrix
must rely upon analysis algorithms (such as those
derived using chemometrics) in order to reveal
information that is specific for one or more chemical
constituent.  Chemometrics techniques can be used to
15    compare unknowns with calibrated standards and data
bases to provide advanced forms of cluster analysis,
and to extract features from an unknown sample that
can be used as information in statistical and
mathematical models.
20            "Principal components analysis" (PCA) is one
method of data reduction which can be performed in the
application of chemometric techniques to spectroscopic
measurement of chemical analytes in a complex matrix.
PCA is used to reduce the dimensionality of a large
25    number of interrelated variables while retaining the
information that distinguishes one component from
another.  This reduction is effected using an
eigenvector transformation of an original set of
interrelated variables (e.g., an absorption spectrum)
30    into a substantially smaller set of uncorrelated
principal component (PC) variables that represents
most of the information in the original set.  The new
set of variables is ordered such that the first few
retain most of the variation present in all of the
35    original variables.  *See, e.g.*, Jolliffe, L.T.,
*Principal Component Analysis*, Sprinter-Verlag, New
York (1986).  More particularly, each PC is a linear

-9-

combination of all the original measurement variables.
The first is a vector in the direction of the greatest
variance of the observed variables. The succeeding
PCs are chosen to represent the greatest variation of

5    the measurement data and to be orthogonal to the
previously calculated PC. Therefore, the PCs are
arranged in descending order of importance.

The term "weighting constant" includes the
wavelength coefficients of partial least squares

10   regression and/or principal components regression, or
any constant obtained from any statistical calibration
that can be used to calculate values (such as analyte
concentration) for unknown samples. A "wavelength
weighting factor" is an embodiment of a weighting

15   constant which is used in the construction of an
optical filter means capable of emphasizing
wavelength-specific information from spectral data.
The wavelength-specific information can be used to
determine desired values relating to the sample

20   undergoing analysis (e.g., analyte concentration). A
wavelength weighting factor can be embodied as a
particular filter density (e.g., neutral or
wavelength-specific), filter thickness, or the like,
such parameters having been determined using the

25   above-described statistical calibration techniques.

The term "optical transfer cell" encompasses
any optically active element that partially absorbs
incident radiation in the visible, ultraviolet, or
infrared spectral regions, wherein the partial

30   absorption is selective with respect to wavelength.
For the purposes of the present invention, an optical
transfer cell generally comprises an optical filter
means having absorption characteristics that were
derived from a partial least squares or principal

35   components regression analysis. The optical filter
means is used to selectively emphasize wavelengths
having high correlation with a selected analyte

concentration. "High correlation," or "cl se
correlation" refers to the quantitative association
b tween the absorption spectrum at a particular
wavelength and a particular analyte concentration,

5      wherein the two variables have a correlation
coefficient (r) of 0.9 or higher.

A "positive correlation filter" is an
optical filter means having an absorption spectrum
sufficient to emphasize radiation of particular

10     wavelengths corresponding to the target analyte and
not to other absorbing analytes. Thus, the positive
correlation filter provides an optimal transfer
function that is highly correlated with the analyte
concentration in the sample being measured. An ideal

15     positive correlation filter would correlate perfectly
with a target analyte (i.e., the correlation
coefficient r would be +1.0), and not correlate at all
with all other interfering absorbing analytes in a
particular sample (r would be 0.0). The synthesis of

20     positive correlation filters is carried out herein
using chemometric techniques to determine appropriate
wavelength weighting factors.

A "neutral density filter" refers to a
standard optical filter means having a flat absorption

25     spectrum. A neutral density filter can be used in
concert with correlation filters in a filter system to
provide a weighting factor to attenuate absorbances
due to the analyte at selected wavelengths and further
improve the accuracy of the correlation provided by

30     the system. A neutral density filter can have an
absorption spectrum sufficient to attenuate radiation
equally at all wavelengths in the range of interest.

As used herein, an "aqueous medium"
encompasses any substrate relating to, made from, or

35     comprising water. Thus, an aqueous medium includes
media wherein water is the major component, i.e., is
present in an amount of at least about 50%, as well as

-11-

wherein water is a solvent but is present in amounts
of less than about 50%.  Aqueous mediums are
sp cifically defined herein to include mammalian
tissue.

5          The term "blood analyte" refers to a blood
constituent that is absorbing in the near-IR range,
the measurement of which is useful in patient
monitoring or in the provision of health care.

          As used herein, the term "near infrared" or
10   "near-IR" encompasses radiation in a spectrum ranging
from approximately 660 nm to 3500 nm, but typically in
the range of approximately 1050 to 2850 nm, and more
typically in the range of approximately 1100 to about
2500 nm.

15          The term "background absorption" relates to
the overall or base level of optical absorption of an
aqueous sample which is to be analyzed, from which the
absorption of a selected constituent departs at one or
more characteristic wavelengths to an extent
20   indicative of the concentration of the selected
constituent.  When the level of background absorption
is high in relation to the characteristic absorption
of the selected constituent, such as in complex
aqueous media where numerous interfering constituents
25   are found, accurate measurement of the magnitude of a
slight change in the absorption at the characteristic
wavelength of a constituent of interest requires
application of the chemometrics techniques described
herein.  This is particularly so in applications
30   wherein the overall concentration of the constituent
of interest is low relative to the aqueous medium,
e.g., in the measurement of blood analytes.


General Methods

35          A spectrophotometric method is provided for
determining the concentration of an analyte in a
liquid sample using near-IR radiation.  In contrast to

-12-

prior techniques, the present method uses all of the
spectral information contain d in the near-IR region
in order to obtain a set of measurements that can be
used to det rmine an analyte concentration with a
5    heightened degree of accuracy.

The method includes the steps of (1)
selecting several distinct, nonoverlapping regions of
wavelengths in the near-IR, wherein each region
defines a spectral range, (2) irradiating a sample
10   using near-IR light containing the selected spectral
ranges to obtain spectrally modified radiation which
has been attenuated, (3) collecting and measuring the
intensity of the spectrally-attenuated radiation at
one or more wavelengths contained within each of the
15   selected spectral ranges, and (4) correlating those
measurements to obtain a value indicative of analyte
concentration.

Spectral information obtained using this
method can be subjected to a combination of
20   mathematical transformations to arrive at a precise
analyte concentration value. For example, standard
statistical techniques, such as partial least squares
(PLS) analysis, or principal components regression
(PCR) analysis, can be used to correlate the
25   absorbance of radiation at specific wavelengths to
analyte structure and concentration. PLS techniques
are described, for example, in Geladi et al. (1986)
*Analytica Chimica Acta* 185:1-17. For a description of
PCR techniques, reference may be had to Jolliffe,
30   L.T., *Principal Component Analysis*, Sprinter-Verlag,
New York (1986).

Accordingly, in determining blood analyte
concentration from a body tissue sample, one method
involves the selection of three nonoverlapping regions
35   of wavelengths from the near IR range spanning 1100 to
3500 nm; specifically, a first region spanning 1100 to
1350 nm, a second region spanning 1430 to 1450 nm or

-13-

1930 to 1950 nm, and a third region spanning 2000 to
2500 nm, wherein each region defines a "spectral
range." The first region contains wavelengths in
which proteins and other cellular components exhibit
5    dominant spectral activity, the second region is
dominated by the absorption spectrum of water, and the
third region contains wavelengths in which organic
analyte molecules exhibit significant spectral
activity. These constituents also contribute to the
10   absorption spectra in those regions where they are not
the dominant species. Accordingly, the spectrally
attenuated radiation obtained from each region
contains a large amount of interrelated information
that must be reduced using statistical methods to
15   obtain analyte-specific information.

The invention also involves the use of
signal processing to improve the accessibility of
physically significant information in the analytical
signals. The intensity values of signals obtained at
20   particular wavelengths can thus be processed to reduce
the effect of instrumentation noise. The processed
signals are then subjected to multivariate analysis
using known statistical techniques.

The PCA method of data reduction is one
25   preferred method used in the practice of the invention
to reduce the dimensionality of a large number of
interrelated variables while retaining information
that distinguishes one component from another. Data
reduction is carried out using an eigenvector
30   transformation of an original set of interrelated
variables (e.g., the absorption spectrum) into a
substantially smaller set of uncorrelated principal
component (PC) variables that represents most of the
information in the original set. The new set of
35   variables is ordered such that the first few retain
most of the variation present in the original set.

-14-

The principal component vect rs can be
transformed by orthogonal rotation against an average
value for the absorbance to obtain both a known
wavelength and the relative value of the absorbance at
5       that wavelength which is attributable to the analyte.
By performing this analysis on information obtained
from each of the three spectral regions, cross-
correlating the principal component vectors via a
linear algorithm, and using subtractive methods to
10      remove the effect of interfering analytes, values are
obtained which can be used in a system algorithm to
determine the concentration of the analyte.

        Multivariate techniques are used to provide
a model that relates the intensity of radiation at
15      specific wavelengths in each spectral region to
analyte concentrations in a particular sample matrix,
e.g., body tissue.  The model is constructed using two
sets of exemplary measurements that are obtained
simultaneously, the first set of measurements, the
20      "prediction set," comprising spectral data, e.g.,
radiation intensity at selected wavelengths, and the
second set of measurements, the "calibration set,"
comprising highly accurate analyte concentrations that
have been determined using invasive sampling
25      techniques.  The procedure is carried out over a range
of analyte concentrations to provide calibration and
prediction data sets.

        Measurements obtained in both the
calibration set and the prediction set are subjected
30      to multivariate analysis, such as by the use of
commercially available multivariate model developing
software programs, to provide an initial model.  The
initial model is applied to the prediction data to
derive analyte concentration values that can be
35      compared to the values obtained by the invasive
techniques.  By iteratively performing the above
steps, a refined model is developed which can be used

to establish a system algorithm for use in analyzing
data obtained by the methods of the invention.

The above-described multivariate techniques
can also be used to design an optically active element
5    capable of enhancing correlation of spectral
information with analyte concentration, e.g., a
positive correlation filter system. Particularly, the
solutions obtained using multivariate analysis can be
used to determine optical parameters, such as
10   absorption characteristics, for positive correlation
filter systems.

In the practice of the invention, non-
analyte specific information from the various
nonoverlapping spectral regions is also used, for
15   example, to normalize each spectral scan, to subtract
background and base line interferences, or to provide
signal values used to detect an inaccurate
measurement.

When determining a blood analyte
20   concentration in a body tissue sample, measurements
taken in the spectral range spanning approximately
1320 - 1340 nm provide a highly reflected,
unattenuated, signal, as there are no major absorption
bands present in the region. By collecting and
25   measuring the intensity of radiation in that range, a
value is obtained which can be used to estimate the
actual intensity of the near-IR light used to
irradiate the sample. The value can be used to
normalize each individual scan and to correct for
30   fluctuations in the intensity of the light source
which could effect the accuracy of analyte
concentration values obtained using the method of the
invention.

Additionally, measurements taken in the
35   spectral ranges spanning approximately 1430 - 1450 nm
and approximately 1930 - 1950 nm provide substantially
non-reflected, highly attenuated, signals, as a result

-16-

of the two dominant absorption peaks occurring at
about 1440 and 1935 nm in the near-IR absorption
spectrum for water.  By collecting and measuring the
intensity of radiation in one or both of those ranges,

5    a value is obtained which can be used to estimate the
intensity of near-IR light that is not totally
absorbed by the irradiated sample.  The value can be
used to subtract background or base-line information
from the analyte-specific signals obtained in other

10   regions and/or to provide an internal reference to
detect inaccurate measurements.  The value can be
subtracted from each spectral measurement obtained
using the present method in order to correct for the
pedestal effect caused by specular reflection which

15   varies with skin texture and age.
            Measurements of substantially unattenuated
signals obtained from a first region (e.g., the
spectral range spanning approximately 1320 - 1340 nm)
and measurements of highly attenuated signals obtained

20   from a second region (e.g., the spectral ranges
spanning approximately 1430 - 1450 nm and
approximately 1930 - 1950 nm) can also be used to
compare diffusely reflected radiation with specular
radiation.  If the signals in the two regions have

25   relatively comparable values, it is likely that most
of the radiation used to irradiate the tissue sample
was reflected from the skin surface, and thus failed
to penetrate the skin to interact with the blood
analytes.  This information can be used to identify

30   ineffective measurements arising from a failure to
obtain a proper instrumentation scan of the tissue
sample.
            The method of the invention can be carried
out using a number of spectrophotometer

35   configurations.  Referring now to Figure 1, one
particular apparatus for determining the concentration
of an analyte in a liquid sample is generally

                              -17-

indicated at **10**.  The apparatus includes a radiation
source **12** which provides a plurality of distinct,
nonoverlapping regions  f wavelengths in the
approximate range of 600 to approximately 3500 nm.  A

5    number of suitable radiation sources are known in the
art, such as incandescent light sources directed
across interference filters, halogen light sources,
modulated by an associated chopper wheel, laser light
sources, laser diode arrays, or high speed light-

10   emitting diode (LED) arrays.  In one particular
apparatus, the radiation source **12** provides radiation
at three distinct regions of wavelengths, specifically
a first region of wavelengths in the near-IR,
typically in the range of approximately 1100 to 1350

15   nm, a second region generally in the range of
approximately 1930 to 1950 nm, and a third region
typically in the range of approximately 2000 to 3500
nm.

The apparatus **10** also includes sample

20   interface optic means **14** which launches incident
radiation from the radiation source into contact with
a sample medium **16** containing an analyte.  After
contacting the sample medium, spectrally modified
radiation emerging from the sample as diffusively

25   reflected light is collected and delivered to a first
lens system **18**, whereby the light is directed into
first and second light paths, respectively indicated
at **20** and **22**.  The first lens system **18** can comprise a
partial reflected mirror configuration such as those

30   known in the art.

In various configurations, the sample
interface optic means **14** can be designed to enable the
close interface of the apparatus **10** with the medium
**16**, such as where the launch is carried out by placing

35   the apparatus in direct contact with the sample
medium, thereby bringing the radiation source into
close pr ximity with the sample to be analyzed.  After

-18-

the launch, the reflected radiation is collected using
optically active means, such as light converging means
or beam deflection optics. Alternatively, the sample
interface optic means 14 can comprise fiber optic
5     waveguides coupled to the apparatus in order to enable
remote apparatus placement and operation. Other
configurations are provided wherein a single fiber
optic bundle is employed to transmit the radiation to
and from the medium. An optrode disposed at the end
10    of the single bundle transmits the near-IR radiation
to the sample medium 16 and receives spectrally
modified radiation therefrom which is directed back
through the bundle to the apparatus 10. Sapphire or
high-grade quartz can be used as optical elements in
15    the above fiber optic waveguides, as those materials
have very good transmission characteristics in the
near-IR spectral range.

The reflected light in the first light path
20 is communicated with a first filter means 22 which
20    has been configured to pass specific wavelengths of
light which are independent of any analyte
concentration. In one configuration, the first filter
means can comprise a narrow band-pass filter which has
near-IR absorption characteristics that selectively
25    pass a region of radiation containing wavelengths with
substantially no correlation with the concentration of
the analyte. Radiation emerging from the first filter
means 22 is then communicated to a first detection
means 24. Communication of the radiation to the first
30    detection means can be carried out via a focusing
means 26, e.g., a collimating lens or the like.
Alternatively, the apparatus 10 can include a
radiation detector that is capable of directly
receiving radiation from the first filter means.
35    The first detection means detects and
converts the passed radiation into a signal that is
representative of the intensity of the analyte-

-19-

independent radiation.  In one particular apparatus,
the first detection means 24 comprises a lead sulfide
photodetector that is able to scan the range of
wavelengths from about 1100 to at least about 3500 nm
5    in steps of 1 nm.

Signals obtained from the first detection
means can be readily converted into digital signals,
e.g, digital signals indicative of the intensity of
the analyte-independent wavelengths, using an
10   analog/digital converter.  The digitized information
is readily available for input into a microprocessor
or other electronic memory means, such as those known
in the art.

Referring still to Figure 1, the reflected
15   light in the second light path 22 is passed to an
adjustable filter means 28 which is capable of having
its absorption characteristics adjusted in response to
a signal that is either externally generated, or that
has been generated by the apparatus 10.  The
20   adjustable filter means generally comprises a screen
filter, such as a neutral density filter, having
absorption characteristics that are adjusted to
variably attenuate the intensity of radiation as
dictated by an external signal or system command.  The
25   degree of attenuation provided by the adjustable
filter means 28 is based upon a predetermined factor
selected to ensure that radiation emitted from the
adjustable filter will be at a constant value
regardless of the intensity of the pre-filtered
30   radiation.  In one particular apparatus, the
attenuation provided by the adjustable filter means is
regulated by a feedback signal generated by the first
detection means 24.

Attenuated radiation emerging from the
35   adjustable filter means 28 is communicated to a
principal analyte filter 30 which has optical
characteristics capable of selectively passing  ne or

-20-

more wavelengths from each of the distinct
nonoverlapping regions of wavelengths launched by the
radiation source **12**.  The wavelengths passed by the
principal analyte filter are selected to have a
5     correlation with the concentration of the analyte.

A second filter means **32** is arranged in the
apparatus **10** relative to the principal analyte filter
**30** such that selectively passed wavelengths emerging
from the principal analyte filter interact with the
10     second filter means, whereby the intensity of each
passed wavelength is independently attenuated by the
second filter means.  The attenuations provided by the
second filter means can be determined, for example, by
an independent set of weighting factors derived using
15     chemometrics techniques.

In one particular configuration, the
weighting factors are determined using a partial least
squares or principal component regression of an
original spectrum obtained from a sample containing
20     the analyte.  The second filter means **32** can be
constructed using a suitable substrate layer that is
capable of transmitting radiation at least in the 600
to 3500 nm range.  The substrate layer is generally
coated with one or more layers of metals and/or oxides
25     that are conventional in the art to provide a
plurality of attenuating filter densities.  Such
coatings can be applied to the substrate using
emulsion or chemical vapor deposition (CVD) techniques
well known in the art.  In an alternative apparatus,
30     the second filter means is a photographic mask having
spectral lines of optical density that are
proportional to weighting functions determined using a
rotated principal components or least squares analysis
technique.
35     After attenuation by the second filter
means, the independent wavelengths are communicated
with a second detection means **34**, such as a PbS

-21-

detector or the like. As described above, the
wavelengths emerging from the second filter means can
be communicated to the second detection means via a
focusing means 36, e.g., a collimating lens or the
5    like. Alternatively, the apparatus 10 can include a
radiation detector capable of directly receiving
radiation from the second filter means.

The second detection means detects and
converts the attenuated wavelengths emitted from the
10   second filter means into a signal which can then be
applied toward an analyte specific algorithm to
determine analyte concentration. Specifically,
signals obtained from the second detection means can
be readily converted into digital signals using an
15   analog/digital converter. The digitized information
is readily available for input into a microprocessor
where it is used to provide an analyte concentration
which can be visualized on a display device and/or
recorded on an output recorder.

20   The apparatus 10 can be used to obtain
measurements of analyte concentration in a variety of
complex media, such as in aqueous media having complex
spectral backgrounds. In one application, the
apparatus can be used in the determination of blood
25   analyte concentrations, particularly organic blood
analytes such as, but not limited to, glucose, urea
(BUN), lipids, bilirubin and alcohol. The blood
analyte can be present in an *in vitro* sample medium
(e.g., a blood sample), or the apparatus can be used
30   to measure blood analytes in tissue. However, the
apparatus 10 is particularly adapted for use in field
applications, e.g., in the measurement of blood
alcohol, or in home health monitoring, e.g., in blood
glucose determination.

35   Referring now to Figure 2, an alternative
apparatus for measurement of analyte concentration in
a complex aqueous medium is generally indicated at 60.

-22-

The apparatus includes a radiation source 62 which
provides a plurality of distinct, nonoverlapping
regions of wavelengths in the approximate range of 600
to 3500 nm.  Radiation from source 62 is transmitted

5   to an optically active means 64 for receiving and
directing the radiation into a beam path and/or for
passing selected wavelengths, such as a collimating
lens, selective filtering means or the like.

Near-IR radiation emerging from means 64 is

10  communicated through a beam splitter 66, whereby the
radiation is divided into two beams, respectively
indicated at 68 and 70.  The first beam 68 from beam
splitter 66 is transmitted to a sample medium 72
containing an unknown concentration of an analyte of

15  interest.  In Figure 2, the sample medium 72 comprises
a sample cell formed from a suitable substrate capable
of transmitting radiation in the near-IR range of
interest.  In one case, the sample can comprise a
blood serum sample, wherein it is desired to determine

20  the concentration of blood analytes.  Alternatively,
the first beam 68 can be transmitted to a sample
surface such as a tissue surface using direct
interfacing means or indirect interfacing means, e.g.,
fiber optic waveguides means such as those described

25  *supra*.  In this manner, the concentration of blood
analytes present in the tissue sample can be non-
invasively determined using reflective near-IR
measurement of the absorption spectrum of radiation
which has interacted with the tissue sample.

30          Radiation, including the spectrally modified
radiation that has interacted with the constituents of
the sample, e.g., the analyte of interest, is then
collected and directed to an optical transfer cell 74
which is disposed in the beam path.  The optical

35  transfer cell 74 comprises a positive correlation
filter system having an absorption spectrum sufficient
to accept the radiation and selectively emphasize one

-23-

or more wavelengths therefrom having high correlati n
with the concentration of an analyte of interest, and
substantially no correlation with interfering
components present in the sample.  The positive

5    correlation filter system thus passes a population of
selected wavelength ranges which provide analyte-
specific information, as well as information about the
measurement background and information that can be
used to correct for instrument changes or interference

10   effects.  Radiation emerging from the optical transfer
cell 74 is received by a detection means 76 for
converting the spectrally modified radiation into a
signal that is representative of the intensity of that
radiation.  The detection means can comprise a broad

15   spectrum photodetector such as an PbS photodetector or
the like.
              Referring still to Figure 2, the second beam
70 from beam splitter 66 is transmitted to an
optically active element 78 which is disposed in the

20   beam path.  In one configuration, the optically active
element 78 comprises a neutral density filter means
having absorption characteristics sufficient to
attenuate radiation equally over a selected range of
near-IR wavelengths.  In an alternative configuration,

25   the optically active element 78 is an optical transfer
cell that includes a positive correlation filter
system having an absorption spectrum that is identical
to the absorption spectrum of optical transfer cell
element 74.  The radiation emerging from the optically

30   active element 78 is received by a detection means 80
for converting the radiation into signals
representative of the intensity thereof.
              The positive correlation filter systems can
be formed from a single substrate layer having an

35   optically active coating which imparts absorption
characteristics capable of selectively emphasizing one
or more wavelengths having high correlation with a

-24-

particular analyte concentration. In particular
system configurations, the positive correlation filter
comprises a plurality of filter layers, each layer
having a sel cted filter density and/ r filter
5    thickness suitable to provide a desired absorption
characteristic. In one case, at least one layer of
the system has a filter density and/or thickness that
comprises a wavelength weighting factor means, wherein
the weighting factor provides enhanced positive
10   correlation of a passed wavelength with the
concentration of an analyte in the selected sample
medium.

The signals generated by detection means **76**
and **80** are then communicated to a means **82** for
15   converting those signals into a digital signal
indicative of the ratio of the intensity of the
radiation emerging from the source **62** and the
corresponding spectrally modified radiation emerging
from the sample. In this manner, changes in the
20   intensity of the radiation emerging from the source **62**
can be corrected for, eliminating a potential source
of error in the measurement obtained from the system.
Further, the ratio of the signals can then be
converted to digital form and interpreted to determine
25   analyte concentration using an internal microprocessor
**84** system or an associated system using methods known
in the art.

If desired, the microprocessor can be
programmed to calculate analyte concentration by
30   application of a chemometrics algorithm to the ratio
signal. The appropriate algorithm can be determined
using the above-described chemometrics techniques,
such as a least squares analysis or rotated principal
component analysis of an original absorption spectrum
35   of the analyte of interest.

-25-

It is to be understood that while th
invention has been described in conjunction with
preferred specific embodiments thereof, the foregoing
description, as well as the examples which follow, are
5    intended to illustrate and not limit the scope of the
invention.  Other aspects, advantages and
modifications within the scope of the invention will
be apparent to those skilled in the art to which the
invention pertains.
10

## Example

A noninvasive glucose measurement was
obtained using the method of the invention.
Particularly, reflective optical measurements in the
15   near-IR region of approximately 1100 nm to 3500 nm
were carried out.  Spectral scans were collected from
volunteer forearm subjects, using an instrument having
a Tungsten-Mercury (W-Hg) radiation source, a Lead
Sulfide (PbS) detector and a scan speed of nm / 0.4
20   second.

A number of specific spectral ranges were
identified as containing information which can be used
to determine glucose concentration from a forearm
tissue scan.  The specified regions were determined
25   from an *in vivo* glucose tolerance study conducted in
tandem with invasively-obtained *in vitro* blood glucose
concentration determinations.  In particular, time-
dependent scans taken during the *in vivo* tolerance
study are depicted in Figure 3.  As can be seen,
30   significant changes in the reflective intensity
differences over the range of about 2120 to 2180 nm
were recorded during the time course of the study.
These changes increased in direct relation to
increases in blood glucose level during the tolerance
35   test, signifying that the range of 2120 to 2180 nm
contains glucose-specific spectral information.

Once the specific spectral ranges were
identified, noninvasive glucose measurements were
obtained using information from the four distinct
spectral ranges. The first spectral range included

5      radiation occurring at about 1320 to 1340 nm. This
range provides a very highly reflected signal, and
there is no major glucose absorption band in this
range. Information obtained from the first spectral
range can be used to normalize each individual scan in

10     order to correct for fluctuations in the radiation
source, and changes due to mechanical perturbations.

The second spectral range included radiation
occurring at either about 1440 to 1460 nm, or about
1940 to 1960 nm. These ranges provide a substantially

15     non-reflected signal due to the highly absorptive
water bands which attenuate diffusively reflected
radiation. Information obtained from these ranges can
be used for
background and base line subtraction from other

20     measurements. These measurements allow for a pedestal
adjustment to account for fluctuations induced by
specular reflection signal values, and can be used to
detect improper measurements.

The third range included radiation occurring

25     at about 1670 to 1690 nm. This range provides
analyte-specific information due to the presence of
glucose vibrational overtone bands.

The fourth range included radiation
occurring at about 2120 to 2280 nm. This range

30     provides analyte-specific information due to glucose
combination vibrational bands.

Signals obtained from the first range were
used to normalize signals of other regions. This
process, when repeated with each spectral scan,

35     eliminates the problem associated with light source
changes and serves to provide an internal reference.
Measurement variations induced by differences in

-27-

optical interface, e.g., patient placements, were
accordingly substantially reduced.

     Background information was eliminated by
subtracting the signals obtained in the second range,
5    from the signals obtained in the third and fourth
analyte-specific ranges.  In this manner, the pedestal
effect created by specular reflection, which varies
with skin texture and age, was corrected for.

     The normalized and base line corrected
10   signals from the third and fourth ranges were applied
in an analytical chemometric analysis.  Figure 4
depicts the normalized differences between signals in
the second and third ranges.

     As can be seen by the results depicted in
15   Figure 4, increase in blood glucose level results in
an increase in the signal differences between the two
ranges.


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Claims:

    1.    An apparatus for determining the
concentration of an analyte in a sample, comprising:

5        (a)    means for irradiating the sample with
incident radiation containing a plurality of distinct,
nonoverlapping regions of wavelengths in the near-IR;

        (b)    means for collecting reflected
radiation emerging from the sample and directing said
10   reflected radiation into first and second light paths,
wherein the first light path comprises radiation from
a first region of wavelengths;

        (c)    first filter means disposed in the
first light path, wherein the first filter means is
15   capable of selectively passing radiation having
substantially no correlation with the concentration of
the analyte;

        (d)    first detection means for receiving
selectively passed radiation emerging from the first
20   filter means, and means for converting the same into a
signal representative of the intensity of said
radiation;

        (e)    adjustable filter means disposed in the
second light path, wherein the adjustable filter means
25   attenuates the intensity of radiation in the second
light path;

        (f)    principal analyte filter means capable
of receiving attenuated radiation emerging from the
adjustable filter means, and selectively passing one
30   or more independent wavelengths therefrom, wherein the
said one or more independent wavelengths are
specifically correlated with the concentration of the
analyte;

        (g)    second filter means capable of
35   receiving the one or more independent wavelengths
emerging from the principal analyte filter means and

-29-

attenuating the intensity of each independent
wavelength;

(h)    second detection means for receiving
the attenuated independent wavelengths emerging from
5    the second filter means; and

(i)    means for converting the detected
wavelengths into a signal representative of the
intensity of said wavelengths.

10            2.    The apparatus of claim 1, wherein the
wavelengths of the incident radiation are in the range
of approximately 1100 to 3500 nm.

              3.    The apparatus of claim 1, wherein the
15    first filter means comprises a narrow band-pass
filter.

              4.    The apparatus of claim 3, wherein the
adjustable filter means comprises a neutral density
20    filter.

              5.    The apparatus of claim 4, wherein the
signal obtained from the first detection means is used
to regulate attenuation provided by the adjustable
25    filter means.

              6.    The apparatus of claim 1, wherein the
second filter means comprises a neutral density
filter.
30

              7.    The apparatus of claim 6, wherein the
attenuation provided by the second filter means is
established using weighting factors.

35            8.    The apparatus of claim 7, wherein the
weighting factors are derived using chemometrics
techniques.

9. The apparatus of claim 8, wherein the weighting factors are derived using rotated principal components analysis of an absorption spectrum f the analyte.

5

10. The apparatus of claim 1, wherein the sample comprises body tissue and the analyte comprises an organic blood analyte.

10

11. The apparatus of claim 10, wherein the blood analyte is selected from the group consisting of glucose, urea (BUN), lipids, bilirubin and ethyl alcohol.

15

12. The apparatus of claim 10, wherein the blood analyte is glucose.

13. An apparatus for determining the concentration of an analyte in a sample, comprising:

20

(a) a source capable of emitting near infrared radiation containing a plurality of distinct, nonoverlapping regions in the near-IR;

(b) means for dividing radiation emerging from the source of part (a) into first and second beam

25 paths;

(c) means for irradiating the sample with the radiation in the first beam path, thereby providing reflected radiation;

(d) means for collecting the reflected

30 radiation emerging from the sample and directing said reflected radiation into a reflected light path;

(e) a first optical transfer cell disposed in the reflected light path, said first cell comprising first positive correlation filter means

35 having absorption characteristics adapted to accept the reflected radiation and emphasize one or more wavelengths from the reflected radiation, wherein said

-31-

one or more wavelengths have high correlation with th
concentration of the analyte in the sample;

   (f)   means for receiving the one or m re
emphasized wavelengths from the first optical transfer
5  cell and for converting the same into signals
representative of the intensity of said emphasized
wavelengths;

   (g)   a second optical transfer cell disposed
in the second beam path, said second cell comprising
10 neutral density filter means having absorption
characteristics sufficient to attenuate the intensity
of the radiation from the second beam path equally
over a selected range of near infrared wavelengths;

   (h)   means for receiving attenuated
15 radiation from the second optical transfer cell and
for converting the same into signals representative of
the intensity thereof; and

   (i)   means for calculating the concentration
of the analyte in the sample using the signals
20 generated by parts (f) and (i).


   14.   The apparatus of claim 13, wherein the
wavelengths of the incident radiation are in the range
of approximately 1100 to 3500 nm.
25

   15.   The apparatus of claim 14, wherein the
second optical transfer cell comprises second positive
correlation filter means having absorption
characteristics identical to those of the first
30 positive correlation filter means.


   16.   The apparatus of claim 14, wherein the
means for calculating the concentration of the analyte
in the sample converts the signals generated by parts
35 (f) and (i) into a digital signal indicative of the
ratio of the intensity of the radiation emerging from

the source and corresponding radiation emerging from the sample.

17.  The apparatus of claim 14, wherein the means for calculating the concentration of the analyte in the sample comprises means for applying a chemometrics algorithm to the signals generated by parts (f) and (i).

18.  The apparatus of claim 13, wherein the first positive correlation filter means comprises a plurality of layers, each layer having selected absorption characteristics such that said filter means emphasizes a population of wavelengths having high correlation with the analyte concentration.

19.  The apparatus of claim 18, wherein the absorption characteristics of at least one layer from the first and second positive correlation filter means are established using weighting factors.

20.  The apparatus of claim 19, wherein the weighting factors are derived using chemometrics techniques.

21.  The apparatus of claim 19, wherein the weighting factors are derived using rotated principal components analysis of an absorption spectrum of the analyte.

22.  The apparatus of claim 13, wherein the sample comprises body tissue and the analyte comprises an organic blood analyte.

23.  The apparatus of claim 22, wherein the blood analyte is selected from the group consisting of

-33-

glucose, urea (BUN), lipids, bilirubin and ethyl
alcohol.

24.   The apparatus of claim 22, wherein the
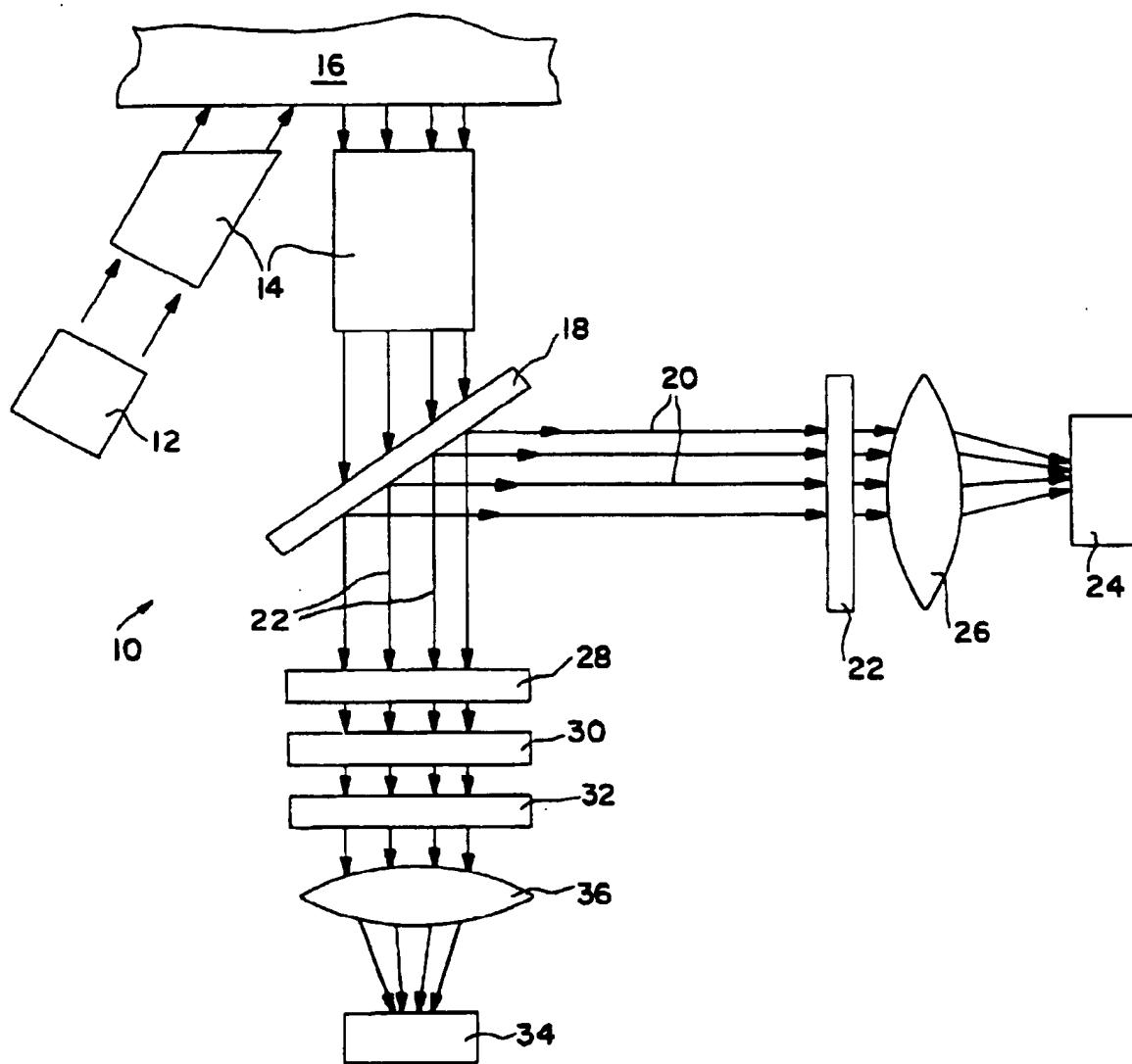5    blood analyte is glucose.
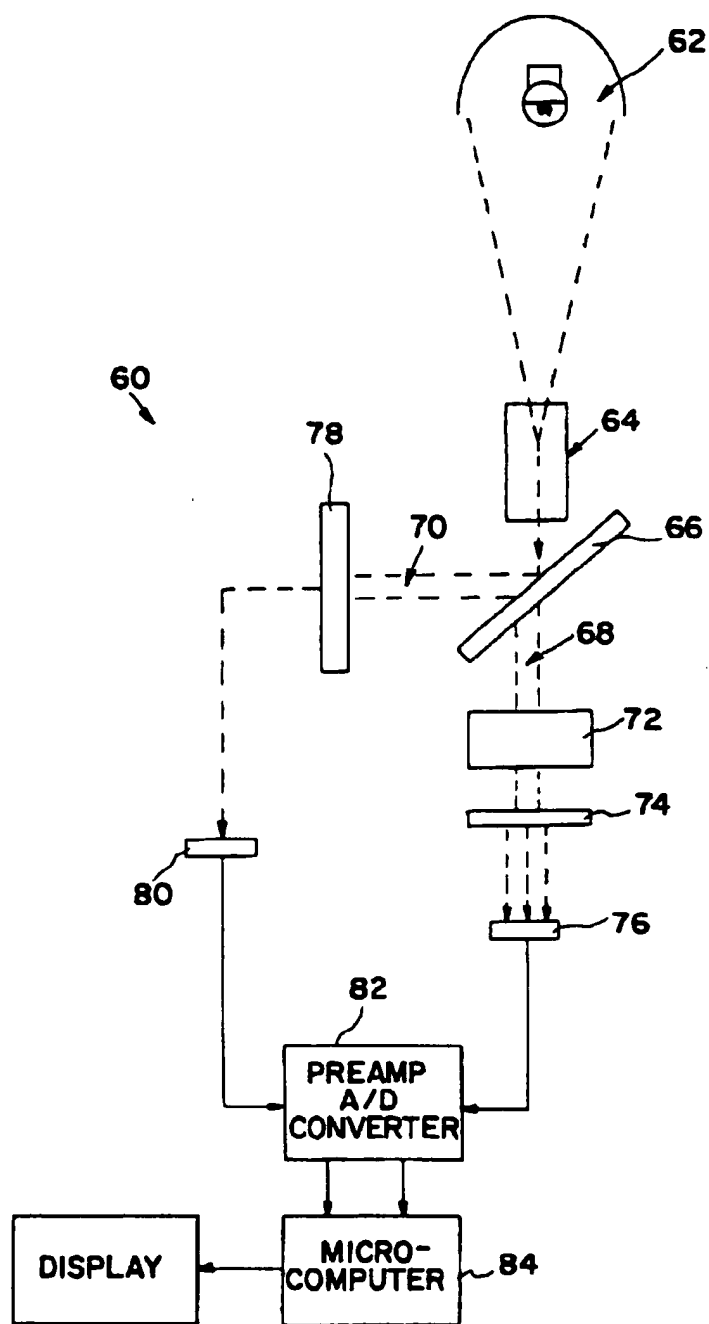
10

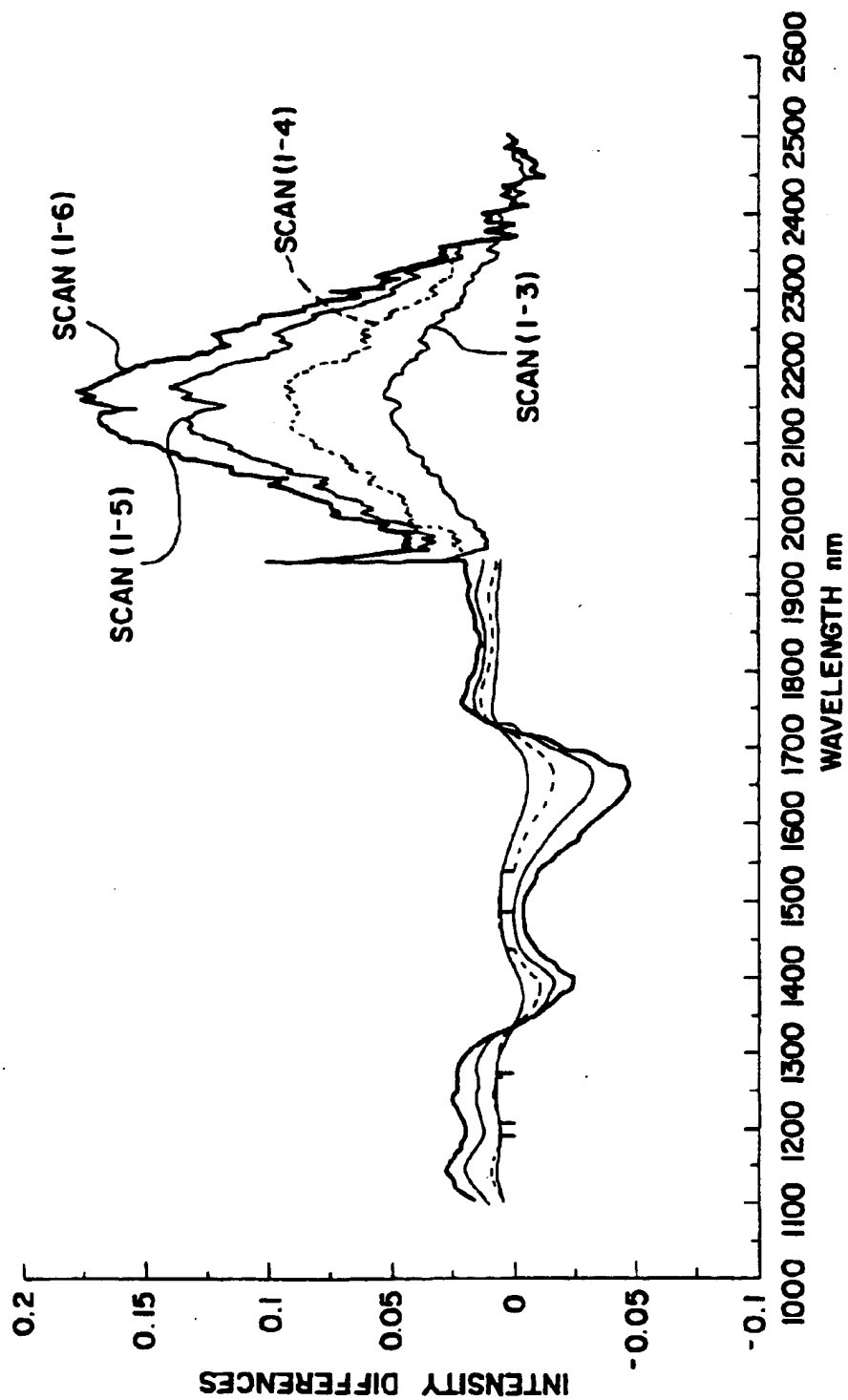15

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-34-

FIG. 1

# FIG. 2
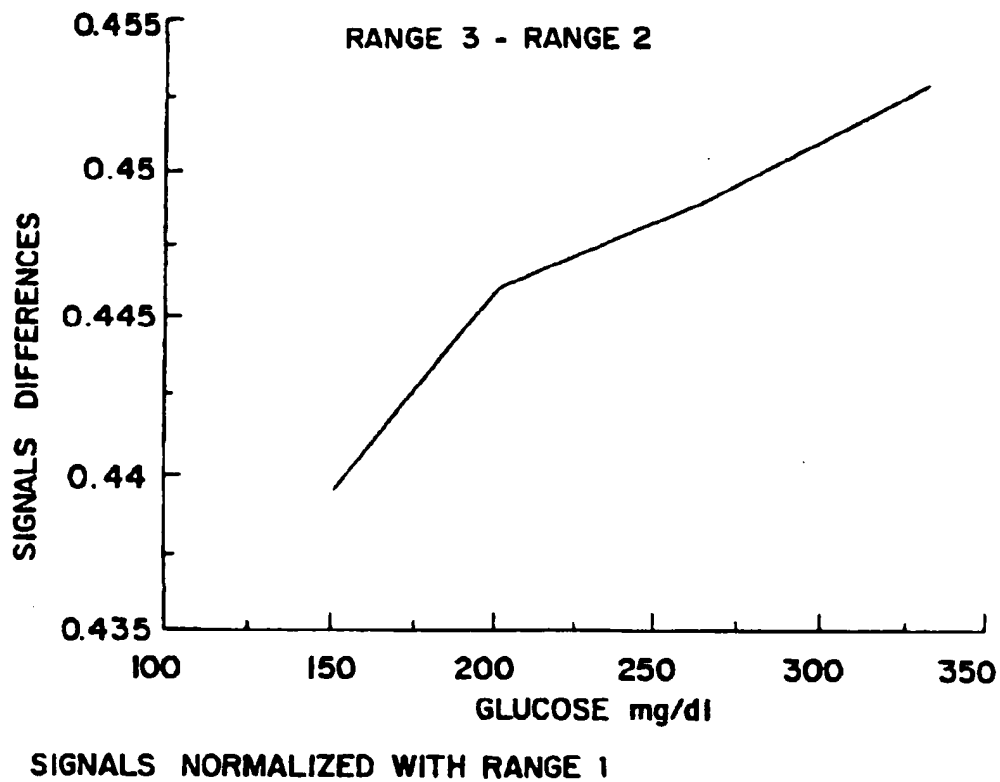
FIG. 3

FIG. 4

# INTERNATIONAL SEARCH REPORT

**A. CLASSIFICATION OF SUBJECT MATTER**
IPC 6    G01N21/35    G01N21/31    A61B5/00

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched  (classification system followed by classification symbols)
IPC 6    G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
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| A | US 3 821 550 A (PRIEST M) 28 June 1974 see column 2, line 33 - line 52 see figure 1 --- | 1 |
| A | US 5 242 602 A (RICHARDSON JOHN  ET AL) 7 September 1993 cited in the application see column 4, line 47 - column 6, line 62 --- | 8,9,17, 20,21 |

-/--

[X] Further documents are listed in the continuation of box C.   [X] Patent family members are listed in annex.

* Special categories of cited documents :

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"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 23 May 1997 | 30. 05. 97 |
| Name and mailing address of the ISA          European Patent Office, P.B. 5818 Patentlaan 2     NL - 2280 HV Rijswijk     Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,     Fax (+31-70) 340-3016 | Authorized officer        Krametz, E |

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**C.(Continuation)  DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
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| E | EP 0 757 243 A (INSTRUMENTATION METRICS INC) 5 February 1997<br>see page 9, line 47 - page 12, line 40<br>see claims; figures<br>----- | 13,14, 22-24 |

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

1

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